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Original Article

EVALUATION OF AQUEOUS EXTRACT OF *CLERODENDRUM COLEBROOKIANUM* ON D-FRUCTOSE INDUCED TYPE 2 DIABETES AND *IN VIVO* ANTI-OXIDANT STUDY ON EXPERIMENTAL ANIMALS

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ABSTRACT

Background

Present study was undertaken to investigate the antihyperglycemic, hypolipidemicand in vivoanti-oxidant effects of aqueous extract of Clerodendrumcolebrookianum (AECC) in experimental diabeticrats.

MATERIALS AND METHODS

The above experiment was carried out with C.colebrookianum extract@ 100 and 300 mg/kg, p.o. for a period of 14 days in diabetic rats. During the experimental period, blood was collected from the fasted rats to determine the blood glucose and lipid profile. Body weight of the animals was recorded on 0^{th}, 7^{th} and 14^{th} day. After the treatment period, lipid peroxidation (LPO), reduced glutathione (GSH), glutathione –S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were also assayed.

RESULTS

Diabetic control rats showed a significant elevation in fasting blood glucose on successive days of the experiment, which was maintained over a period of two weeks.After 14 days treatment with AECC (100 and 300 mg/kg, p.o.) and standard drug (Glibenclamide @ 1 mg/kg), a significant ($P<0.01$) decrease in the blood glucose as well as plasma and liver total cholesterol, triglycerides and phospholipids was observed. Besides, anti-oxidant properties of AECC was also elucidated in this experiment and found that administration of AECC to the diabetic rats caused significant ($P< 0.01$; $P< 0.05$) reduction of liver enzymes viz LPO, GSH, SOD, CAT & GST.

CONCLUSIONS

Our results demonstrated that AECC with its antidiabetic, hypolipidemic and antioxidant properties could be a potential herbal medicine in treating diabetes and other related complications.

KEYWORDS: D-Fructose Induced Diabetes, Clerodendrumcolebrookianum, Plasma and Liver Lipid Profile & Liver Antioxidant Enzymes.

Short Running Title

Activity of Clerodendrumcolebrookianum aqueous extract in diabetic rats.

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INTRODUCTION

Diabetes mellitus is a heterogenous metabolic disorder involving carbohydrate, protein and fat metabolism due to a relative or an absolute lack of insulin. Insulin resistance which is a major factor of type 2 diabetes is the condition in which normal amount of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells. Chronic obesity leads to increased insulin resistance that can develop into diabetes most likely because adipose tissue (especially that in the abdomen around internal organs) is a source of several chemical signal to other tissues (hormones and cytokines). About 55% type 2 diabetes are obese.[1]Thus present experiment was planned to observe both hypoglycemic and hypolipidemic activity of the plant extract in the experimental model.

There is increasing evidence that complications (macro and micro vascular) related to diabetes are associated with oxidative stress, induced by the generation of free radicals.[2] Prolonged hyperglycemia in uncontrolled diabetes mellitus leads to glucose oxidation, which is believed to be the main source of free radicals.[3]

Plant based drugs have been in use against various diseases since time immemorial. The essential values of some plants have long been published but a large number of them remain unexplored as yet. So there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties.[4] *Clerodendrumcolebrookianum*under the family *Verbenaceae*, posse'svastmedicinalproperty in the folklore. The pharmacological studies revealed that the extract from *C.colebrookianum*Walp protects rat heart against oxidative stress induced by ischemic-reperfusion injury (IRI).[5] Fresh juice treatment significantly reduced the SGOT level while ethylacetate extract increased the SGPT level without damaging the liver cells in rats,[6] extract of *C. colebrookianum* increased the antioxidant capacity of blood and had an inhibitory effect on the basal level of lipid peroxidation of liver and kidney.[7]

Though experimental studies indicated anti-oxidant role of *C. colebrookianum* against oxidative stress in experimental animals, but its activity against hyperglycemia and hyperlipidemia is lacking. The present study was therefore, undertaken for systemic evaluation of aqueous extract of *C. colebrookianum* for its anti-diabetic, hypolipidemic and anti-oxidant potential on experimental models.

MATERIALS AND METHODS

Preparation of Extract

For preparing cold aqueous extract, the method used by Manjunatha*et al.,* (2005)[8] was followed with slight modification. One hundred and twenty five (125) grams of powder of *C. colebrookianum* was soaked in 1000 mlof double distilled water for a period of 4 days with intermittent stirring and at the end of 4th day the content was filtered with muslin cloth followed by Whatman filter paper number 1. The extract obtained was further subjected to vacuum evaporation at

(60-70)^{0}C for 24 hours and lyophilized for successive 24 hours. Lyophilization was stopped when the extract appeared sufficiently dry. Further the material was stored at -40^{o}C in deep freeze in air tight containers until use.

Phytochemical Screening

Phytochemical tests were conducted on the aqueous extract of *Clerodendrumcolebrookianum* as per standard procedures.[9]

Acute oral Toxicity

The acute oral toxicity studies of aqueous extract of *C. colebrokianum* were undertaken in mice (30 ± 10 grams). The animals were divided into 4 groups with 5 numbers of animals in each group. Group I received the extract of *C. colebrookianum* @ 1000 mg/kg, group II received the extract @ 3000 mg/kg, Group III received the same @ 6000 mg/kg and Group IV received the extract @ 9000 mg/kg. The study was done for 72 hours and during this period the animals were observed for mortality, sign of any abnormality, feeding-watering as well as body weight change.

Induction of Type 2 Diabetes

The blood samples were collected by retro-orbital puncture of the rats and blood glucose was determined before D-fructose administration. D-Fructose (BDH, Poole, England) with a molecular weight of 180.16g was used for the study. Each rat, regardless of weight, consumed a solution containing 6.6gof fructose/5ml of distilled water (through an oral cannula) daily for30 days. All animals were maintained under the same laboratory conditions of temperature (ambient temperature maintained between 26-28°C), humidity and light (L:D; 12:12) and were allowed free access to food and water. After 30 days, blood glucose of the animals was monitored and the animals having blood glucose more than the basal value was used for further study.

Experimental Design

Thirty apparently healthy animals (100 ± 20 grams) of either sex were selected for the experiment and divided into five groups of 6 animals each. Group I served as normal diet control (without induction of hyperglycemia) and received normal diet while in Groups II, III, IV and V hyperglycemia was induced by allowing the animals to feed high fat and carbohydrate diet for about 30 days. Following induction of hyperglycemia, Group II received normal saline as treatment, Group III received aqueous extract of *C. colebrookianum* @ 100 mg/kg, p.o. and Group IV received aqueous extract of *C. colebrookianum* @ 300 mg/kg, p.o.while Group V received standard drug i.eGlibenclamide @ 1 mg/kg, p.o.

Biochemical Estimation

During the experimental period, blood was collected from 12 hour fasted rats by means of capillary tube through orbital sinus. Blood glucose level was estimated by auto analyser using a commercial assay kit (Quantum Biologicals Pvt. Ltd.). Likewise plasma and liver total cholesterol, triglycerides and phospholipids was estimated by using various commercial assay kits.

At the end of the experimental period, the animals from each experimental group were sacrificed by cervical dislocation and blood was processed for further biochemical estimation. Tissue such as liver was collected in ice-cold containers and homogenized in 10% physiological saline. The homogenate was centrifuged at 15,000 X g for 30 min at 4^0 C and the supernatant was used for the determination of superoxide dismutase (SOD) activity by the method of Marklund

and Marklund (1974)[10], catalase (CAT) activity by the method of Sinha (1972)[11], glutathione-S-transferase (GST) activity by the method of Habig*et al*. (1959)[12] and reduced glutathione (GSH) activity by the method of Ellman (1959)[13].

Statistical Analysis

The values of above parameters were expressed as mean ± SEM. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnettes multiple comparison tests. $P< 0.05$ was considered to be statistically significant.

RESULTS

Phytochemical Screening

The extract of *C. colebrookianum*was qualitatively analyzed for the presence of different phytochemical constituents. The extracts were found to contain tannins, terpenoids, saponin, flavonoid and cardiac glycosides but phlobatannins and steroids were found absent.

Acute oral Toxicity

Aqueous extract of *C. colebrookianum*@ 1000, 3000,6000and 9000 mg/kg, p.o.did not produce any mortality or apparent sign of overt toxicity during the period of observation for 3 days. Therefore, the extract was found to have LD_{50} above 9000 mg/kg. However at 9000mg/kg, some behavioral changes like depression and reduced alertness were observed.

Evaluation of C. Colebrookianum Extracts for Hypoglycaemic Properties

The effect of aqueous extracts of *C.colebrookianum*on D-fructose induced type 2 diabetes in rats is shown in Figure 1 (a). Administration of aqueous extracts at 100 and 300 mg/kg, p. o. significantly ($P<0.01$) reduced (120.38±2.27 and 110.16±0.47 mg/dl) the D-fructose induced hyperglycaemia as compared to the vehicle control (263±0.72 mg/dl). However the reduction of blood glucose level was highest (94.16±1.17 mg/dl) and significant ($P<0.01$) in standard treated group with glibenclamide @ 1 mg/kg.

During induction of diabetes with D-fructose, there was reduction of body weight in all the experimental groups except the normal control group (non inducing diabetes). Soon after the treatment with the extract and standard drug, the body weight of the animals regain and received to a normal level as compared to diabetic control group. The results are summarized in Figure1 (b).

Evaluation of C. Colebrookianum Extract on Lipid Profile of Diabetic Rats

There was a significant rise in plasma and liver total cholesterol, triglyceride and phospholipids level in D-fructose induced diabetic rats. After 14 days treatment with *C.colebrookianum* extract @ 100 and 300 mg/kg and glibenclamide @ 1 mg/kg, there was significant ($P< 0.01$) reduction of plasma total cholesterol from 148.16±2.02 to 85.66±1.93 (100 mg/kg) and 81.50±1.53 (300 mg/kg) as compared to diabetic control rats (148.16±2.02). However, the reduction was highest in standard treated group with a value of 76.33±1.46. Likewise, there was significant ($P< 0.05$; $P< 0.01$) reduction of liver cholesterol, plasma and liver triglycerides and plasma and liver phospholipids in the extract treated as well as standard treated groups as compared to diabetic control rats. The results were summarized in Table 1.

Evaluation of C.Colebrookianum Extract for in- Vivo Anti-Oxidant Parameters

Effect of *C. colebrookianum* extract on enzymatic antioxidants like LPO, SOD, CAT, GST and GSH in liver was

estimated and the values are given in Table 2. The concentration of LPO in liver increased in the diabetic rats (18.26±1.03 m moles/100g tissue) when compared to normal control rats (11.07±1.02 m moles/100g tissue). The administration of *C.colebrookianum* extract @ 100 and 300 mg/kg could significantly ($P< 0.05$) lower the concentration of LPO in liver (12.22±1.00 and 11.37±0.58 m moles/100g tissue). However, there was a significant ($P< 0.05$) increase in the activities of SOD (39.56±1.29 and 47.40±1.76 U/mg protein), CAT (22.24± and 24.32± µmoles of H_2O_2 utilized/min/mg protein), GST (208± and 223± nmoles of CDNB conjugated/min/mg protein) and GSH (273± and 310± n moles/100 g tissue) after administration of *C. colebrookianum* extract @ 100 and 300 mg/kg as compared to diabetic control rats. Similar trend of antioxidant activity was also observed in the standard treated group with glibenclamide.

DISCUSSIONS

The present experiment revealed the hypoglycaemic, hypolipidemic as well as antioxidant property of aqueous extract of *C. colebrookianum* on D-fructose induced type 2 diabetic rats. In the present study, rats were fed with a high-fructose diet for a period of four weeks in order to induce insulin resistance. Previous studies have shown that long-term fructose-feeding induces mild insulin resistance in experimental animals.[14,15] Insulin resistance is considered as a part of the metabolic risk profile including central obesity, cardiovascular, hypertension, and diabetes. In the present experiment, aqueous extract of *C. colebrookianum* @ 100 and 300 mg/kg, reduced the blood glucose level significantly ($P< 0.01$) as compared to control value. Like many chronic diseases, chronic hyperglycemia is widely believed to cause elevated concentrations of reactive oxygen species accompanied by lowered enzymatic and nonenzymatic cell antioxidant defences.[16,17] Reactive oxygen species have been suggested to be involved in beta cell dysfunction and insulin resistance.[18] Thus anti-oxidant property of *C.colebrokianum* extract was also investigated and found that *C. colebrookianum*extract @ 100 and 300 mg/kg could significantly ($P< 0.05$) lower the concentration of LPO in liver and also produced significant ($P< 0.05$; $P< 0.01$) increase in the activities of SOD, CAT, GST and GSH as compared to the diabetic control rats. Beneficial effect of *C. colebrookianum* extracts on insulin resistance state may be due to the antioxidant capacity of these compounds. Thus, there is possibility of hypoglycaemic property of *C. colebrookianum*due to its anti-oxidant activity of diabetic rats.

Fructose feeding stimulates the hepatic production of triglycerides, both by promoting the reestrification of circulating non-esterified fatty acids and also by stimulating de novo fatty acid synthesis.[19]Increased delivery of triglycerides or non-esterified fatty acids to the muscle interferes with the utilization of glucose, through the principles of Randle cycle,[20] and also impairs the insulin action. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hyper triglyceridemia[21] and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities.[22] Administration of aqueous extract of *C. colebrookianum* @ 100 and 300 mg/kg, could lower the serum and liver lipid profile significantly towards normal as compared to diabetic control rats. Similar work done by (Devi and Sharma 2004)[6] showed that organic and crude extracts of *C. colebrookianum* significantly lowered the serum lipid profile in rats suggesting that it has cardioprotective potential.

Flavonoids extracted from the fruits of *Solanummelongena* (Brinjal) at a dose of 1mg/100g BW/day showed significant hypolipidemic action in normal and cholesterol fed rats.[23]From phytochemical screeningof *C.colebrookianum*, it was evident that aqueous extract of *C. colebrookianum*contain flavonoidsalong with other phytoconstituents. Thus there is a possibility of lowering plasma lipid profile, after treated by *C. colebrookianum* extract may be due to its flavonoid

content. Further, the effect of *C. colebrookianum* on alloxan induced type-1 diabetes is ongoing for complete evaluation of the extract on diabetic profile.

REFERENCES

1. *Eberhart MS, Ogden C, Engelgau M, Cadwell B, Hedley AA, Saydah SH. Prevalence of overweight and obesity among adults with diagnosed diabetes – United States, 1988-1994 and 1999-2002.Morb and Mort W Rep 2004;53:1066–8.*
2. *Jakus V. The role of free radicals, oxidative stress antioxidant systems in diabetic vascular disease.BratislLekListy 2000;10:541-51.*
3. *King GL, Loeken MR. Hyperglycemia induced oxidative stress in diabetic complications. Histochem cell boil 2004;122:333-8.*
4. *Baquar SR. Medicinal and poisnous plants of Pakistan. Karachi.1989.p.95-96, 184-185, 248-249, 337-440.*
5. *Devi R, Banerjee SK, Sood S, Dinda AK, Maulik SK. Extract from ClerodendroncolebrookianumWalp protects rat heart against oxidative stress induced by ischemic-reperfusion injury (IRI).Life Sci 2005; **77**: 2999-3009.*
6. *Devi R, Sharma DK. Hypolipidemic effect of different extracts of Clerodendrumcolebrookianum Walp in normal and high-fat diet fed rats. J Ethnopharmacol 2004; 90: 63-68.*
7. *Rajlakshmi D, Banerjee SK, Sood S, Maulik SK. Anti-oxidant capacity of Clerodendrumcolebrookianum. J PharmaPharmacol 2003;55: 1681-91.*
8. *Manjunatha BK, Vidya SM, Rashmi KV, Mankani KL, Shilpa HJ, Jagadeesh S. Evaluation of wound healing potency of VernoniaarboreaHk. Ind J Pharmacol 2005;37:223-226.*
9. *Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical Constiuents of some Nigerian medicinal plants.Afri J Biotechnol 2005; 4: 685-688.*
10. *Marklund S, Marklund G. Involvement of superoxide dismutase anion radical in the antioxidant of pyrogallol and a convenient assay for SOD. Eur J BiolChem 1974;47:469-474.*
11. *Sinha AK. Calorimetric assay of catalase.AnnalBiochem 1972;47:389.*
12. *Habig WH, Pabst MJ, Jakoby WR. Glutathione S-transferase: the first enzymatic step in mercapturic acid formation. J BiolChem 1974; 249:7130-7139.*
13. *Ellman GL. Tissue sulphydryl groups. Arch BiochemBiophy 1959;82:70-77.*
14. *Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. NutrMetab2000;2:5.*
15. *D'Angelo G, Elmarakby AA, Pollock DM, Stepp DW. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. Hypertension 2005; 46:806–811.*
16. *Asem, Bidyarani. "Evaluation of Textual Readability–An Analysis of Its Varying Approaches."*
17. *Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab 2000;26:163–176.*
18. *Catherwood MA, Powell LA, Anderson P, McMaster D, Sharpe PC, Trimble ER. Glucose-induced oxidative stress in mesangial cells.Kid Int 2002; 61:599–608.*
19. *Evans JL, Goldfine ID, Maddux BA, Grodsky, GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes 2003; 52:1–8.*
20. *Southgate DA. Digestion and metabolism of sugars.Am J ClinNutr1995; 62: 203S–211S.*

21. *Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev1998; 14:263–283.*

22. *Pushparaj PN, Low HK, Manikandan J, Tan BK, Tan CH. Anti-diabetes of Chichoriumintybus in STZ-induced diabetic rats. J Ethnopharmacol 2007;111:430-34.*

23. *Laze, Blerta, and Anila Mitre. "Preliminary Evaluation of Chorus System in Comparison with Mini-Vidas System for Detection of Cytomegalovirus-IgM Antibodies."*

24. *Murali B, Upadhyaya UM, Goyal RK. Effect of chronic treatment withEnicostemmalittorale in non insulin dependent diabetic (NIDDM) rats. J Ethnopharmacol 2002; 81:199-204.*

25. *Sudheesh S, Presannakumar G, Vijayakumar V, Vijayalakshmi NR. Hypolipidemic effect of flavonoids from Solanummelongena.Plant FoodHum Nutr1997; 51:321–330.*

Table 1: Effect of *C. Colebrookianum* Extract on Plasma and Tissue Lipids (Liver)

Groups	Treatment	Plasma (mg/dl)			Liver (mg/dl)		
		Total Cholesterol	Tri-Glycerides	Phospho-Lipids	Total Cholesterol	Tri-Glycerides	Phospho-Lipids
I	Normal control	72.66±2.59	47.66±1.65	76.00±2.00	8.66±1.06	10.56±0.82	80.50±1.53
II	Diabetic control	148.16±2.02**	96.50±2.42*	133.16±1.37	15.33±0.96**	14.82±1.21*	150.83±1.04
III	Diabetic+ AECC (100)	85.66±1.93**	77.33±1.98*	83.66±1.93*	10.33±1.31*	11.68±0.72*	91.66±1.15*
IV	Diabetic+ AECC (300)	81.50±1.53**	62.16±1.13*	70.83±1.04*	7.66±1.15**	9.00±1.00**	80.33±1.31*
V	Diabetic+ glibenclamide(1)	76.33±1.46**	64.00±1.67*	72.00±1.18*	7.00±1.00**	10.16±0.82*	79.83±1.13*
All values are expressed as mean± SEM (n=6); Group II is compared with Group I. Groups III, IV and V are compared with group II; $^{*}P< 0.05$; $^{**}P< 0.01$							

Table 2: Effect of *C. Colebrookianum* on Liver Anti-Oxidant Enzyme Activities of Normal and Diabetic Treated rats During the Study

Groups	Treatment	LPO(mmoles/ 100g Tissue)	GSH(mmoles/ 100g Tissue)	SOD(U/mg Protein)	Catalase (U/mg Protein)	GST(U/mg Protein)
I	Normal control	11.07±1.02	314.89±6.6	31.28±1.36	92.03±3.23	44.43±2.29
II	Diabetic control	18.26±1.03*	182.47±7.3**	16.44±1.55**	63.20±2.35**	32.24±1.51*
III	Diabetic+ AECC (100)	12.22±1.00*	291.68±8.0**	22.80±1.26*	72.45±1.56*	37.04±2.20
IV	Diabetic+ AECC (300)	11.37±0.58**	350.58±5.7**	36.91±1.75**	84.13±2.29**	49.6±2.67**
V	Diabetic+ Glibenclamide (1)	11.50±0.88**	293.51±8.4**	30.53±1.24**	88.87±2.19**	42.82±1.44*
All values are expressed as mean± SEM (n=6); Group II is compared with Group I. Groups III, IV and V are compared with group II; $^{*}P< 0.05$; $^{**}P< 0.01$						

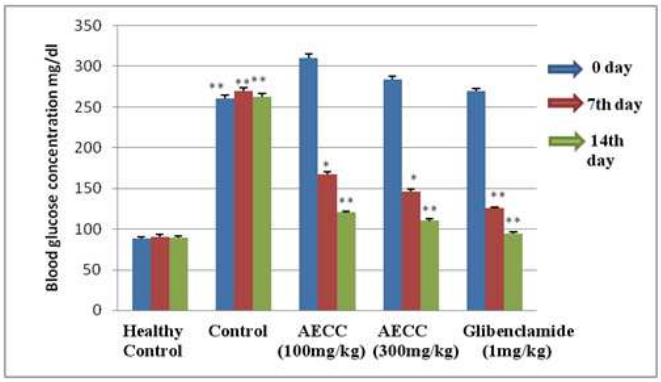

Figure 1(a)

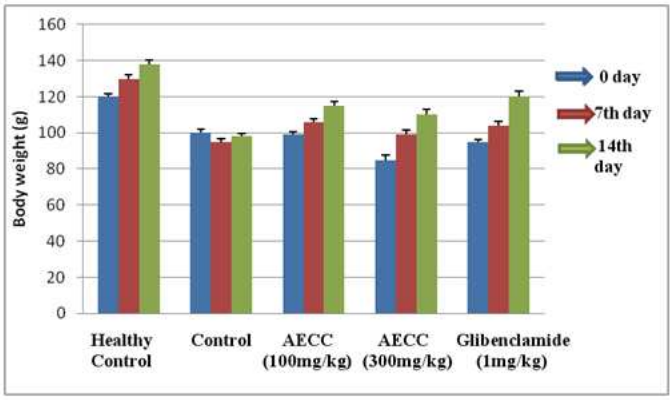

Figure 1 (b)

Figure 1: Blood glucose (a) and mean body weight (b) of normal, diabetic untreated and diabetic treated groups during the experimental period. All values are expressed as mean± SEM (n=6); Group II is compared with Group I. Groups III, IV and V are compared with group II $^{*}P< 0.05$; $^{**}P< 0.01$